MOLECULAR WEIGHT DETERMINATION BY CHROMATOGRAPHY ON SEPHAROSE 4B

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1. Introduction

The use of cross-linked dextran gels (Sephadex) for the determination of molecular weights of proteins is generally accepted. Moreover, gel filtration chromatography is the only simple and generally applicable method for the determination of molecular weights of biological active proteins in impure preparations. From the known experimental data Determann and Michel [1] showed that the relationship between the elution volume (V_e) and the logarithm of the molecular weight (M) of globular proteins, in the linear range of this relation, could be described by the following equations:

G-75: $\log M = 5.624 - 0.752(V_e/V_o)$,

G-100: $\log M = 5.941 - 0.847(V_e/V_o)$,

G-200: $\log M = 6.698 - 0.987(V_e/V_o)$,

(V_o = void volume).

However, for the determination of molecular weights above about 300.000, the dextran gels are useless. Polson [2] introduced gel filtration on granulated agar which permitted fractionation in a range up to several millions. Andrews [3] showed that for chromatography on agar gels the same type of relarion between $\log M$ and V_e holds. Hjertén [4], Bengtsson and Philipson [5] described the preparation and use of agarose gels. The advantage of agarose above agar is the absence of charged groups. These agarose spheres are now commercially available in several fractionation ranges.

We used Sepharose 4B (Pharmacia, Uppsala, Sweden) in order to determine the molecular weight of an aminopeptidase from beef spleen earlier described by us [6].

2. Experimental

Several proteins of known molecular weight were chromatographed – in separate runs – on a column which was prepared as recommended by the Pharmacia manufacturer. The chosen compounds were:

A) Hemocyanin of *Helix pomatia*, mol. wt. 9×10^6 [7]
B) Erythrocruorin of *Arenicola marina*, mol. wt. 2.75×10^6 [8]
C) Erythrocruorin of *Planorbis corneus*, mol. wt. 1.3×10^6 [8]
D) Hemocyanin of *Helix pomatia*, 1/10 subunits, mol. wt. 9×10^5 [7]
E) Urease (Serva), mol. wt. 4.73×10^5 [9]
F) Catalase (Boehringer), mol. wt. 2.25×10^5 [10]
G) Immunoglobulin G (rabbit) (Povite), mol. wt. 1.6×10^5 [11]
H) Serumalbumin (bovine) (Povite), mol. wt. 6.7×10^4 [12]
I) Hexokinase (yeast) (Sigma), mol. wt. 4.5×10^4 [13]
J) Trypsin (Worthington), mol. wt. 2.4×10^4 [14]
K) Ribonuclease (bovine) (Seravac), mol. wt. 1.4×10^4 [15]
L) Glycyl-L-glycine (Mann), mol. wt. 132

Fig. 1 shows the diagram obtained after plotting the elution volume V_e (peak position) against the logarithm of the molecular weight (M). As can be seen

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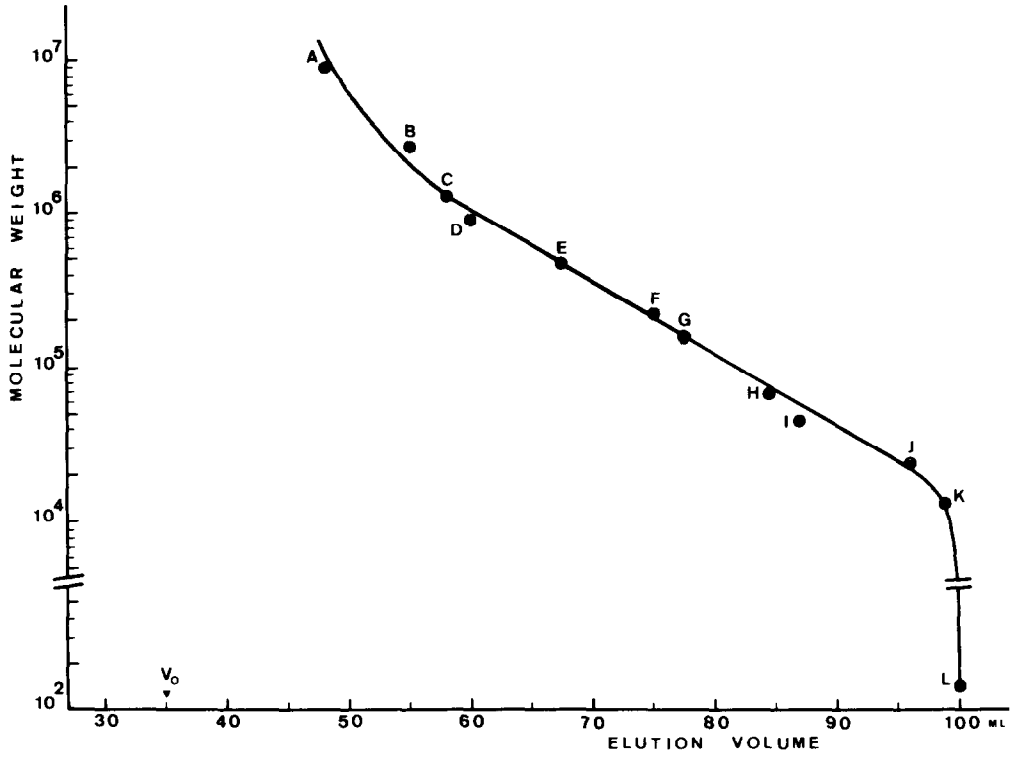

Fig. 1. Molecular weight as a function of the elution volume. The logarithms of the molecular weights of a number of macromolecules – A to L, see text – were plotted against the elution volume (V_e) after chromatography on Sepharose 4B. Bed dimensions: 1.7 X 43.5 cm. Sample volume: 1 ml. Flow rate: 6 ml/h. The samples were dissolved in 0.01 M veronal, 0.10 M NaCl, pH 7.0 (except sample D, which was dissolved in veronal/NaCl, pH 8.3; at this pH 1/10 subunits are present). The column was equilibrated with the buffers mentioned. The elution was followed by measuring the absorption at 280 nm, except for urease (E) where its enzymic reaction (16) was used. The position of the dipeptide was determined by the ninhydrin reaction (17). V_o, the void volume, was established with dextran 2000 (Pharmacia); the leading peak emerged with an elution volume of 35 ml.

there is a linear relationship over the range 2×10^4 to 1×10^6. From this line we can calculate an equation similar to those of Determann and Michel [1]. For Sepharose 4B, in our hands, the equation becomes:

$$\log M = 8.78 - 1.62(V_e/V_o) . \quad (1)$$

Equation (1) can also be written in the following way:

$$\log M = 7.16 - 3.01 K_d , \quad (2)$$

where K_d, the distribution coefficient, is defined as $K_d = (V_e - V_o)/(V_t - V_o)$, where V_t represents the total volume (given by the elution position of glycylglycine).

Using the given calibration curve we found for the mentioned aminopeptidase [6] a molecular weight of about 300.000.

3. Discussion

As has been observed by other investigators, the choice of buffer, molarity and pH sometimes have great influence on the elution of the compounds by gel filtration chromatography. Although agarose is said to be free of charged groups, we found the elution of several proteins highly influenced when instead of the veronal/NaCl buffer, only the veronal buffer was used as the eluant. Thus hemocyanin,

erythrocruorin of *Arenicola*, catalase, serumalbumin and hexokinase were eluted too early, whereas ribonuclease was not eluted at all unless salt was added. The elution position of trypsin and glycyl-glycine, on the other hand, was not influenced by addition of salt. The void volume as determined with dextran 2000 remained also unaffected. Sedimentation analysis of the proteins showed that the salt effect was not due to molecular weight changes.

In conclusion, Sepharose 4B shows the same relationship between V_e and $\log M$ of proteins as has been found for the cross-linked dextran gels, but one should take care in the choice of the buffer (addition of 0.1 M NaCl is recommended). Sepharose 4B appears to be suitable over a broad molecular weight range.

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